

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Inventors: Van Eyk et al.
Serial No.: 09/115,589
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Examiner: Borgeest, Christina M.
Customer No.: 26259
Group Art Unit: 1649
Confirmation No.: 1553
Title: Methods of Diagnosing Muscle Damage

DECLARATION BY JEREMY A. SIMPSON

I, Jeremy A. Simpson, hereby declare:

1. I am a co-inventor on the above referenced patent application.
2. I have reviewed the Office Action mailed October 4, 2007, and in particular the Examiner's questions relating to data presented in my Declaration submitted July 27, 2007.
3. To address the Examiner's questions and for clarification, I am representing the data of Figures 1A and 1B from my Declaration of July 27, 2007 in separate Figures 1 and 2. Data presented in Figure 2 of my Declaration of July 27, 2007 is now presented in Figure 3.
4. To generate the data presented in Figures 1 through 3, serial serum samples were obtained from patients with various skeletal muscle injuries including trauma, seizures, and drug-induced rhabdomyolysis. Serum proteins were separated by a modified sodium dodecyl sulfate-polyacrylamide gel electrophoresis protocol followed by Western blotting with monoclonal antibodies specific for fsTnI.

5. Figure 1 shows representative Western blots of serial serum samples from 5 patients probed for fsTnI. All 5 patients had been admitted to Kingston General Hospital with indications of skeletal muscle disorders. The blots have been labeled Patient 1 through Patient 5. For this experiment, the first blood sample on hospital admission is considered time 0 and is shown in the left-hand lane of each blot. The lanes of each blot have been labeled with the times at which each serum sample was obtained from each patient, for clarification. As shown in Figure 1, all 5 patients had detectable levels of fsTnI. Patients 1 and 2 had detectable levels of fsTnI degradation products. Under the same conditions, serum samples from healthy individuals (i.e., experimental controls) show no detectable levels of either intact or fragments of fsTnI (data not shown). The proteolytic fragments and fsTnI were detected using anti-fsTnI specific mAb F1-32, commercially available as of the filing date of this patent application from Spectral Diagnostics.

6. Figure 2 shows representative Western blots of serum samples from 6 additional patients. The blots have been labeled Patient 6 through Patient 11. Arrows adjacent to the blots of Patients 6, 9 and 11 highlight at least 7 different proteolytic fragments of fsTnI observed on the basis of migration on 1-dimensional SDS-PAGE. The proteolytic fragments and fsTnI were detected using anti-fsTnI specific mAb F1-32, commercially available as of the filing date of this patent application from Spectral Diagnostics.

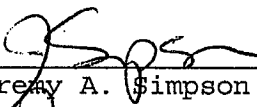
Also shown in Figure 2 is that while the presence and number of proteolytic fragments varied between Patients 6 through 11, the levels were unrelated to creatinine kinase (CK) levels. CK levels for each of Patients 6 through 11 are shown below each blot.

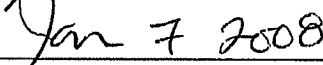
7. Figure 3 shows two Western blots of serial serum samples obtained over a 3 day time period from a single patient suffering from skeletal muscle damage resulting from seizures, probed with two different anti-fsTnI specific mAbs, F1-32, commercially available as of the filing date of this patent application from Spectral Diagnostics, and anti-fsTnI specific mAb SI-1, commercially available as of the filing date of this patent application from Hytest. Each lane of the gels represents a different serum sample obtained from the same patient in time sequential order over the 3 day period. At least two proteolytic fragments with a

molecular weight of about 20 and 25 kDa were detectable in the serum of the patient over time with mAb FI-32 (top panel). Importantly, both proteolytic fragments were detectable with mAb FI-32 (indicating that the fragments are present in the serum) while they were not detectable with mAb SI-1 (bottom panel; indicating the fragments were modified in a way which affects mAb SI-1 binding) over the first day and a half of hospital stay (lanes 1-4).

8. Thus, as shown by Figures 1 through 3, we have detected multiple proteolytic fragments of fsTnI in serum of human patients suffering from various skeletal muscle disorders using two different anti-fsTnI specific mAbs commercially available as of the filing date of this patent application. The proteolytic fragments were detected in serum via SDS-PAGE and Western Blot Analysis similarly to the procedures set forth at pages 28-30 of our patent application. Any modifications in procedure were routine changes due to, for example, differences in sample type.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.



Jeremy A. Simpson


Date

Figure 1

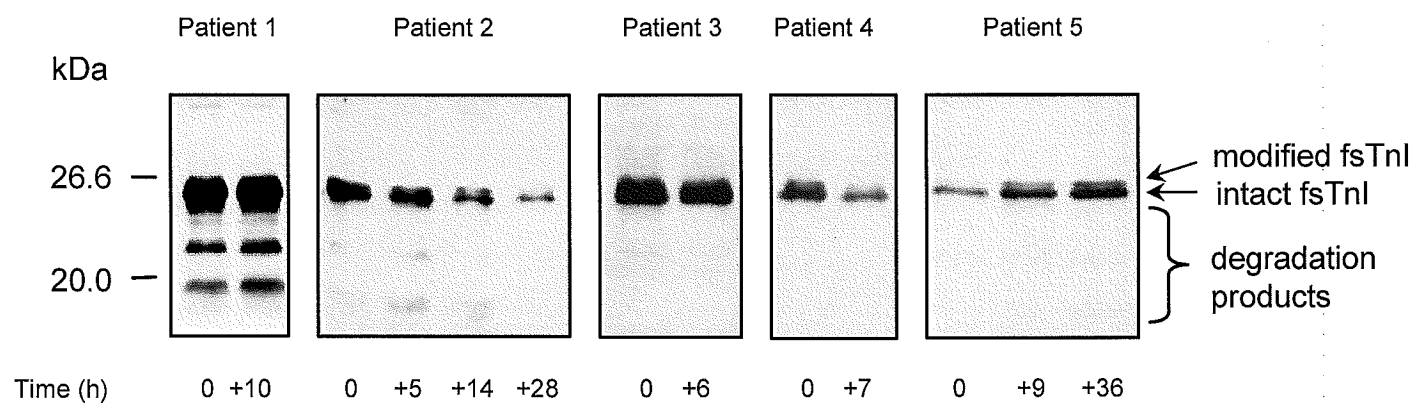


Figure 2

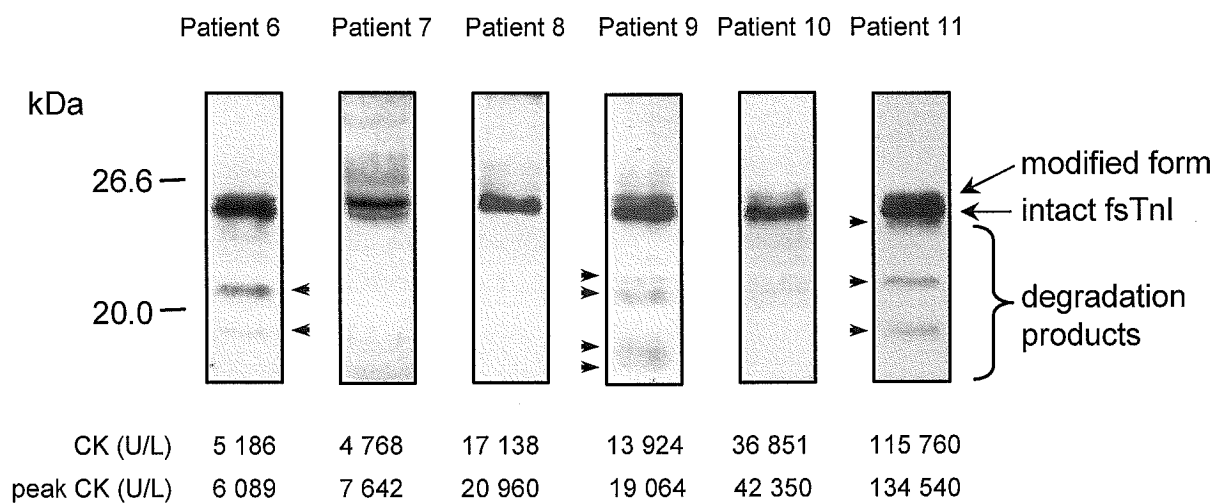


Figure 3

